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AF 1644  
JFW  
PATENT -- FEE JFW

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE  
BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES

In Re: Application of	)	
PETER NASH ET AL	)	
	)	
Serial No.: 09/616,843	)	
	)	
Filed: July 14, 2000	)	Group Art Unit 1644
	)	
For: IMMUNOGEN ADHERENCE INHIBITOR	)	Exr. P. Huynh
AND METHOD OF MAKING AND	)	
USING SAME	)	
	)	
Case Docket No.: C150.12.3B	)	

**LETTER**

Commissioner for Patents  
P.O. Box 1450  
Washington, D.C. 20231

Sir:

Enclosed are the original and three (3) copies of Appellants' Reply to Examiner's Answer  
including Exhibits A to G; and

A small business statement is of record.

Respectfully submitted,

PETER NASH ET AL

By Richard O. Bartz  
Richard O. Bartz  
Registration No. 20,468  
Southdale Office Centre  
6750 France Avenue South, Suite 350  
Edina, MN 55435-1983  
(952) 920-3959



hereby certify that this correspondence is being deposited with the United States Postal Service  
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Alexandria, VA 22313-1450 on JUNE 7, 2004,  
(Date of Deposit)

RICHARD JOHN BARTZ

Name of applicant, assignee, or Registered Rep.

R. John Bartz

Signature

JUNE 7, 2004

Date of Signature



PATENT -- FEE

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USING SAME	)	
	)	
Case Docket No.: C150.12.3B	)	

**APPELLANT'S REPLY TO EXAMINER'S ANSWER**

Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

Sir:

The application contains Claims 14, 15, 16, 19 to 24 and 27 to 32 attached as Appendix to the Examiner's Answer. These claims define a method of promoting the growth of food animals. The method includes egg IgY immunoglobulins that bind to the protein-wasting immunogens to inhibit adherence of the protein-wasting immunogens in the intestinal tracts of animals. The result is that the binding of the immunogens blocks adhesion and colonization of the immunogens which compliments the animal's natural biological systems to maximize feed efficiency and growth.

The claims are rejected as unpatentable under 35 USC 103(a) based upon a combination of a number of prior art references.

35 U.S.C. 103(a) Conditions for Patentability

"A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject

matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains."

The requirement of 35 U.S.C. 103(a) is that the subject matter as a whole "would have been obvious" to a person having ordinary skill in the art. In other words, the claimed method and beneficial result would have been produced by the combination of the teachings of the prior art.

The Examiner in the Examiner's Answer has introduced a new issue with regard to the requirement of 35 U.S.C. 103(a). The Examiner concludes "[f]rom the combined teaching of the references, it is apparent that one of ordinary skill in the art would have had a reasonable expectation of success in producing the claimed invention." *Examiner's Answer, page 8, lines 4-6; page 10, lines 17-19; page 15, lines 15-17; Page 26, lines 3-5; Page 29, lines 27-29 and page 23, lines 9-11.* A "reasonable expectation" is not the requirement of 35 U.S.C. 103(a). A reasonable expectation of producing the claimed invention suggests that the claimed invention may or may not be produced. The 35 U.S.C. 103(a) requirement is not a maybe yes or maybe no or speculation analysis with regard to producing the claimed invention. Under 35 U.S.C. 103(a) the claimed invention must be produced from the teachings of the prior art.

The rejection of Claims 14, 15, 16, 19 to 24 and 27 to 32 as unpatentable based on 35 U.S.C. 103(a) must be reversed in view of the Examiner's erroneous application of the requirements of 35 U.S.C. 103(a) and the insufficient teachings of the prior art noted in Appellant's Brief.

Enclosed is a publication "*The effect of dietary chicken egg-yolk antibodies on clinical response in weaned pigs challenged with a K88<sup>+</sup> Escherichia coli isolati.*" J. Swine Health Prod. 204:12(3): 119-122. The study reported in this publication states that the egg-yolk-antibody IgY product did not protect susceptible pigs from developing diarrhea as a result of oral challenge

with *E. coli*. This study follows the teaching of *Tokoro* which is the primary prior art reference in the rejection of the claims. The results noted in this publication are contrary to the Examiner's contention of a reasonable expectation of producing the claimed invention. It also is evidence that the claimed invention is not obvious to a person of ordinary skill in the art.

Appellants have discovered that egg IgY immunoglobulins must bind to protein-wasting immunogens to inhibit adherence of the immunogens in the intestinal tracts of animals. The totality of the teachings of the prior art do not reveal this discovery and advantageous results.

Appellants have conducted bead studies to demonstrate that antibodies disclosed in the application bind to bacteria. Bead studies were used because you can see them more clearly. The beads are activated and then coated with antibodies from specific egg products disclosed in the application. The following Exhibits are part of the bead studies.

Exhibit A are uncoated plastic beads.

Exhibit B shows egg-coated beads with no bacteria.

Exhibit C shows *Streptococcus bovis* bound to a bead coated with antibody taken directed from rumen fluid.

Exhibit D shows *E. coli* plus a bead with normal egg.

Exhibit E shows *E. coli* O157:h7 bound in three dimensions to O157 antibody coated bead.

Exhibit F shows *Fusobacterium necrophorum* bound to two beads.

The binding action of the egg immunoglobulins to Appellant's claimed method promoting the growth of food animals is a discovery beyond the teachings of the prior art.

In summary, (1) the Examiner has not applied the correct requirements of 35 U.S.C. 103(a) in the rejection of Claims 14, 15, 16, 19 to 24 and 27 to 32 and has allowed a person skilled in the art to speculate with regard to producing the claimed invention and advantageous

results of promoting the growth of food animals and (2) Appellant's have discovered a method of promoting the growth of food animals that includes egg immunoglobulins that bind to protein-wasting immunogens with the result that the immunogens are inhibited from adherence to the intestinal tracts of animals and have demonstrated the binding action of the egg immunoglobulins.

Appellants request that the final rejection of the claims be reversed and that Claims 14, 15, 16, 19 to 24 and 27 to 32 be allowed.

Respectfully submitted,

PETER NASH ET AL

By Richard O. Bartz  
Richard O. Bartz  
Registration No. 20,468  
6750 France Avenue South, Suite 350  
Edina, MN 55435-1983  
(952) 920-3959

EXHIBIT A

Beads





**EXHIBIT B**

**Egg+Béads**

**s Bov**

EXHIBIT C

Streptococcus Bovis

A black and white micrograph showing a dense population of Streptococcus Bovis bacteria. The bacteria appear as numerous small, dark, circular or oval-shaped structures, many of which are arranged in chains or small clusters. The background is a light, grainy texture, likely representing the growth medium. The text "Streptococcus Bovis" is overlaid in the center-left area of the image.

**EXHIBIT D**

E-coli on normal egg bead

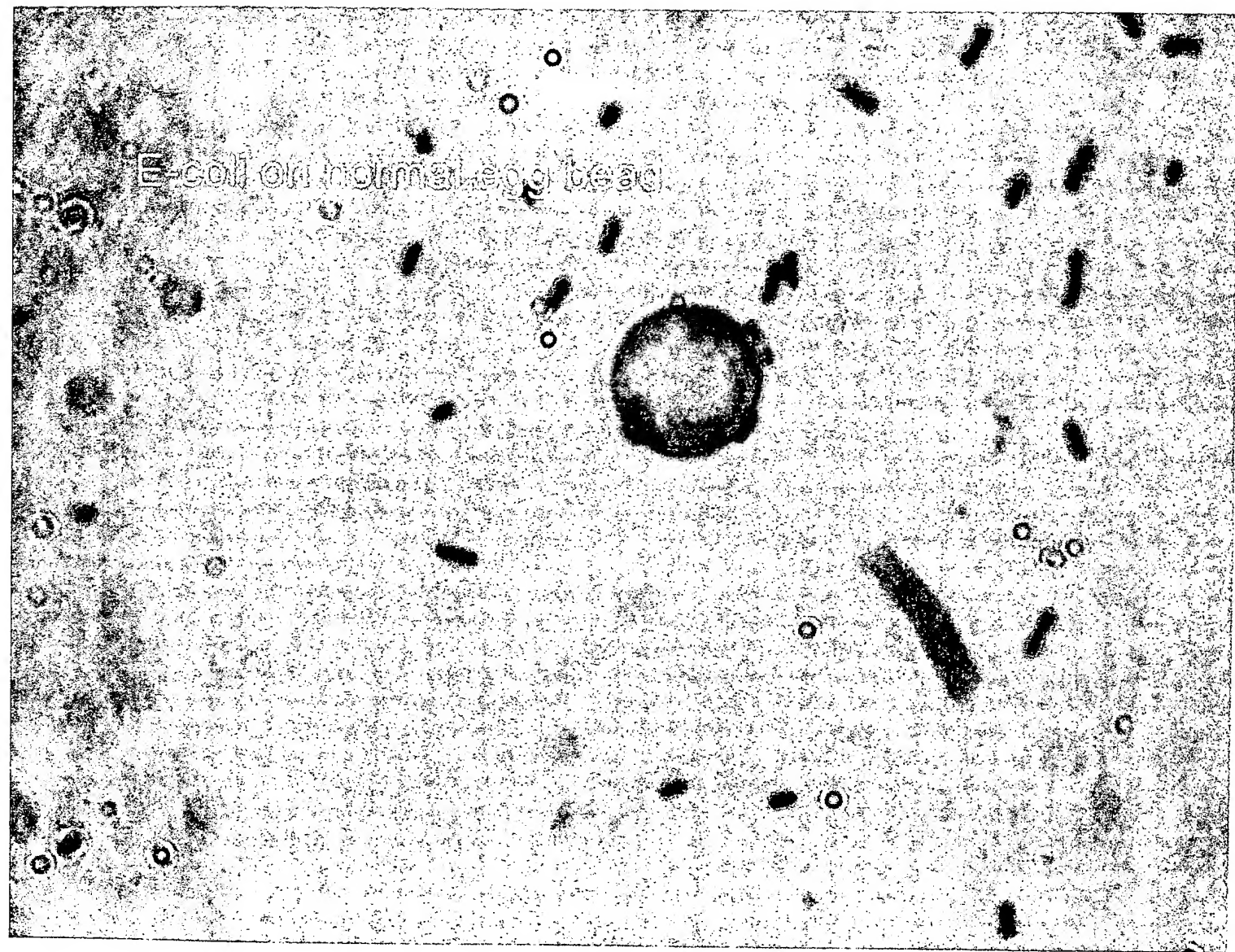


EXHIBIT E

E-coli 0157:H7

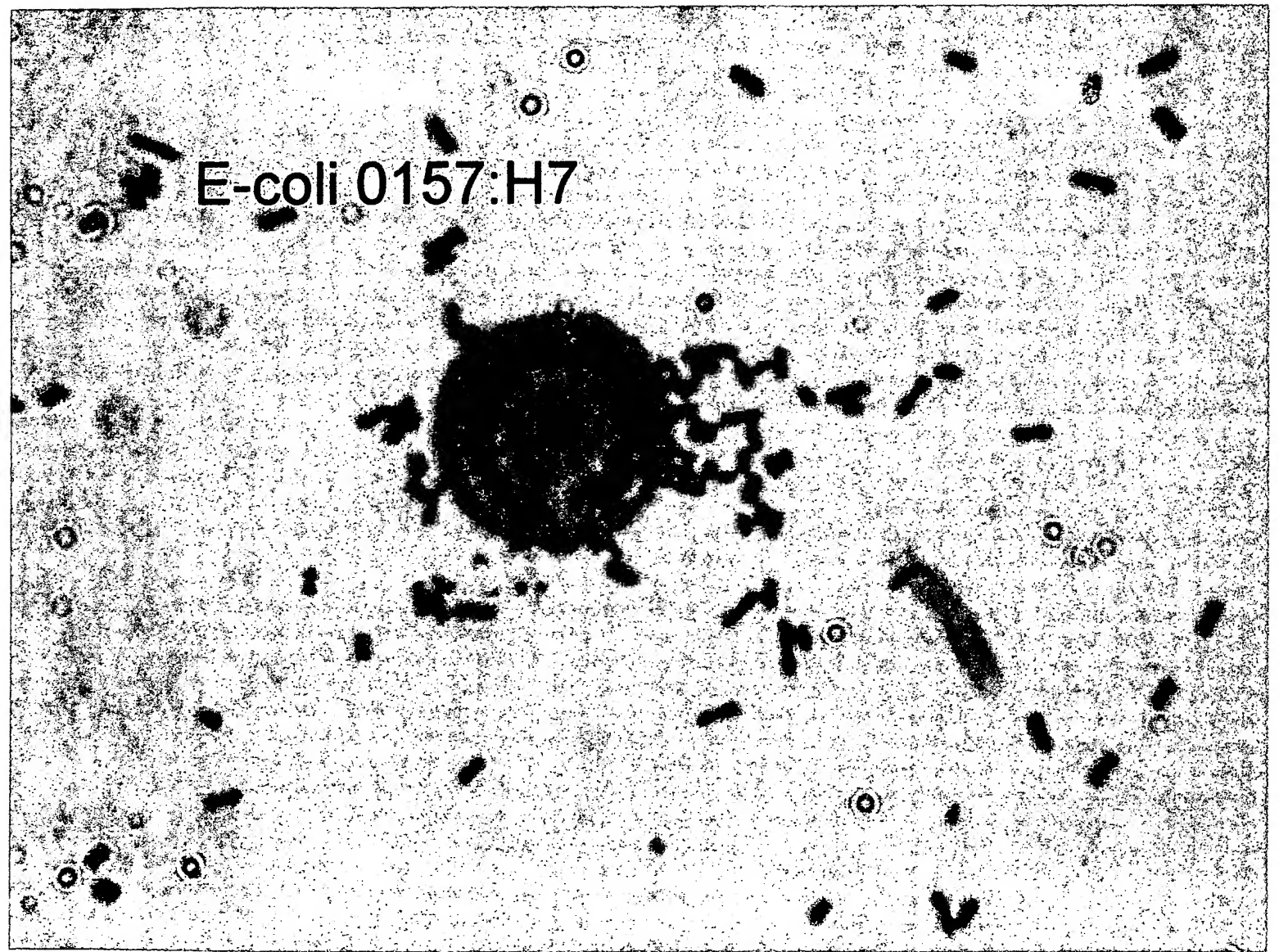




EXHIBIT F



*Fusobacterium necrophorum*

This electron micrograph shows a single, large, dark, rod-shaped bacterium, identified as *Fusobacterium necrophorum*. The bacterium has a thick, textured outer layer and a slightly tapered end. It is surrounded by a field of smaller, circular, electron-lucent structures, which appear to be cellular debris or other microorganisms. The background is a grainy, light gray, typical of electron micrographs.

# The effect of dietary chicken egg-yolk antibodies on the clinical response in weaned pigs challenged with a K88<sup>+</sup> *Escherichia coli* isolate

Larisa V. Chernysheva, DVM, MSc; Robert M. Friendship, DVM, MSc, Diplomate ABVP; Catherine E. Dewey, DVM, MSc, PhD; Carlton L. Gyles, DVM, PhD

## Summary

**Objectives:** To determine if supplementing the diet of weaned pigs with a specific chicken egg-yolk-antibody product would protect against experimentally induced colibacillosis.

**Methods:** Four treatment groups of 12 newly weaned pigs (approximately 22 days old) were fed either a control diet (Groups 1 and 2) or the same diet containing chicken egg-yolk antibodies (IgY) at inclusion rates of 0.32% (Group 3) and 3.2% (Group 4). Pigs in Groups 2, 3, and 4 were challenged with 5 mL of a suspension containing  $10^{11}$  colony forming units per mL of a K88<sup>+</sup> isolate of *Escherichia coli*.

**Results:** Pigs in Group 1 (unchallenged controls) did not develop diarrhea. In the three groups challenged with *E. coli*, pigs developed watery diarrhea and dehydration. Prevalence and severity of clinical signs were similar in all three groups. No appreciable levels of IgY were detected either in treated or control pigs by ELISA testing of small intestine content.

**Discussion:** The *E. coli* challenge was successful in creating a clinical response similar to field cases. The presence of chicken egg-yolk antibodies in the ration did not appear to be effective in preventing the disease. It is possible that IgY activity is greatly reduced by gastric acid and pepsin,

and therefore, even at high inclusion rates, egg-yolk antibodies may not be efficacious in pigs as old as 3 to 4 weeks of age.

**Implications:** Supplementation of chicken egg-yolk-antibody products may not be effective in controlling postweaning *E. coli* diarrhea.

**Key words:** swine, postweaning mortality, diarrhea, passive immunity

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Postweaning *Escherichia coli* diarrhea is an important cause of economic loss for pig producers.<sup>1</sup> Often, as a response to postweaning diarrhea, farmers incorporate antibiotics and high concentrations of zinc oxide in starter rations. However, concerns have arisen with regard to the emergence of antimicrobial resistance and to the environmental impact of heavy metals in manure. A variety of alternative approaches have been tried with disappointing results.<sup>2</sup> Treatments that have been investigated include acidification of feed or water, probiotics, prebiotics, and vaccination.

One alternative technique that has shown potential as an inexpensive and effective control measure for postweaning *E. coli* diarrhea is the use of chicken egg-yolk antibodies (IgY). Chickens that have been vaccinated against specific enterotoxigenic *E. coli* (ETEC) fimbrial antigens produce high levels of IgY in their eggs. Products made from the yolks of eggs from immunized chickens have been administered orally to piglets and offer prophylactic and therapeutic value in controlling colibacillosis.<sup>3</sup> However, in field studies, morbidity and mortality appeared to be unaffected, possibly because of the interaction of environ-

mental and management factors and the presence of concurrent diseases.<sup>4</sup> Possibly a better method to determine the value of chicken egg-yolk antibodies in the control of postweaning diarrhea is by using a controlled experimental *E. coli* challenge study. However, some researchers have reported that experimental infection to create clinical disease is difficult to achieve.<sup>5</sup>

The objectives of this study were to determine whether an oral challenge using a K88<sup>+</sup> ETEC isolate could induce diarrhea in postweaned pigs as old as 3 to 4 weeks of age, and to determine if egg-yolk antibodies were protective against an experimentally induced *E. coli* infection.

## Material and methods

All challenge studies were approved by the University of Guelph Animal Care Committee and were in accordance with the Canadian Council on Animal Care guidelines for the care and use of experimental animals.

LVC, RMF, CED: Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

CLG: Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario.

Corresponding author: Dr Robert M. Friendship, Department of Population Medicine, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1; Tel: 519-824-4120, ext 54022; Fax: 519-763-3117; E-mail: rfriends@ovc.uoguelph.ca.

This article is available online at <http://www.aasv.org/shap.html>.

Chernysheva LV, Friendship RM, Dewey CE, et al. The effect of dietary chicken egg-yolk antibodies on the clinical response in weaned pigs challenged with a K88<sup>+</sup> *Escherichia coli* isolate. *J Swine Health Prod.* 2004;12(3):119-122.

## Animals

Forty-eight purebred Yorkshire pigs from the University of Guelph's swine research farm were weaned at approximately 3 weeks of age (mean = 22.0 days, SD = 3.4 days) and assigned randomly to one of four treatment groups. All pigs appeared healthy, with no history of diarrhea, but their carrier status for ETEC was not evaluated prior to the study.

## Experimental design

A total of 48 pigs were used in the three replicates of the trial, with 12 pigs allotted to each of four treatment groups in the study. In each trial, 16 pigs were assigned to four pens, with each pen representing a treatment group. At the time of arrival at the isolation facility, pigs were given an ear tag for identification purposes and weighed.

Pigs in Groups 1 and 2 were fed a control diet (a standard starter ration containing no antibiotics). Pigs in Groups 3 and 4 received a similar diet except for addition of egg-yolk-antibody product. The feed for Group 3 was prepared by including the egg-yolk-antibody product at the level recommended by the manufacturer (3.2 g per kg or 0.32% of the total diet) and for Group 4 the egg-yolk-antibody product was added at 10 times the recommended level (32 g per kg or 3.2% of the total diet). Pigs were allowed to acclimatize to the facility for 3 days. During this time, experimental diets were offered *ad libitum*, and all pigs were observed to be eating the feed.

On the third day, pigs were challenged with a preparation of *E. coli* isolated from a field case of postweaning colibacillosis. Five mL of a suspension containing  $10^{11}$  colony forming units (CFU) per mL was given by gavage to each pig in Groups 2, 3, and 4. Group 1 was not challenged with *E. coli* (negative control).

The experimental diets were offered *ad libitum* until the end of the experiment. Pigs were observed hourly postchallenge for development of clinical signs, including lethargy, inappetence, dehydration, gauntness, and diarrhea. Pigs with severe watery diarrhea were euthanized. Thirty-six hours after challenge, all remaining pigs were euthanized and necropsy examinations were performed.

## Egg-yolk-antibody product

The egg-yolk-antibody product, obtained from Dr Ron Marquardt (Department of

Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada), contained antibodies specific to *E. coli* fimbrial antigens K88, K99, and 987P. The method used for preparation and purification of the egg-yolk antibodies was similar to that described by Kim et al (1999).<sup>6</sup> The powdered egg-yolk-antibody product was mixed with mash feed by hand because of the small quantities of feed used in these trials.

## Challenge culture

A strain of O149 K88ac<sup>+</sup> *E. coli* isolated from a field case of postweaning diarrhea was used to challenge the pigs. In order to prepare sufficient inoculum, the *E. coli* was streaked onto a blood agar plate. One colony was transferred to 15 mL of brain-heart infusion broth and incubated for 18 to 24 hours. Six 4-L baffle flasks were prepared with 800 mL of brain-heart infusion broth, and to each flask, 2.5 mL of the inoculated broth was added. The flasks were placed on a platform shaker (INNOVA; Brunswick Scientific, Edison, New Jersey) and rotated at 150 rpm overnight at 37°C. A 1-mL sample of broth was diluted logarithmically and plated on blood agar to determine the number of CFU per mL. The bacteria were pelleted by centrifugation and resuspended in phosphate buffered saline (PBS) to a concentration estimated to be  $5 \times 10^{11}$  CFU per 5-mL dose.

## Collection of intestinal samples

Euthanasia was performed by intravenous injection of pentobarbital at a dosage of 70 mg per kg of body weight. Fecal swabs collected from all pigs at euthanasia were submitted to the Animal Health Laboratory, University of Guelph (Guelph, Ontario, Canada), for culture and typing of *E. coli* isolates. Contents of the stomach, small intestine, and large intestine were diluted 1:6 in PBS and homogenized. Final pH of stomach content was 7.2 to 7.4. Each sample was centrifuged at 1000g for 5 minutes, and the supernatants were filtered through 0.45 µm membrane filters<sup>6</sup> and stored at -20°C until the assay for IgY was performed.

## ELISA for IgY

A direct ELISA was used to detect anti-K88<sup>+</sup> IgY immunoglobulins in feed and gastrointestinal samples. Microtiter plates were coated with K88 antigen. The procedure for the ELISA is described by Jin et al (1998).<sup>7</sup> A sample size of at least 2 g was

used for the initial (ie, highest) dilution to obtain the best results. Antibodies were diluted to such a degree that the plate reader could analyze the wells without exceeding its absorption spectrum. For example, the recommended dilution is 1:2000 for egg-yolk antibodies and 1:10 for feed samples.

## Statistical analysis

A general linear model analysis of variance procedure (GLM) (SAS Institute Inc, Cary, North Carolina) was used to determine the effect of different dosages of antibodies at initial and final time of sampling. Least squares means were computed for each dosage time and their interaction. Descriptive statistics for numerical data and test for normality were conducted with PROC UNIVARIATE in SAS program (PC-SAS 6.12; SAS Institute).

A mixed linear model (SAS PROC MIXED; SAS Institute) was used to model treatment group effects on the titre of IgY K88 antibody in the gastrointestinal tracts of pigs, while controlling for the random effects of pig nested within the fixed effects of the treatment nested within the random effects of trial. Tukey's HSD test (PROC GLM, SAS/STAT) was applied in all pairwise comparisons of means following detection of significant interaction effects on intestinal samples. Descriptive statistics for numerical data and tests for normality were conducted with PROC UNIVARIATE in SAS. All multivariate models were run in SAS PROC MIXED. Interaction effects were included in all models and all variables were tested. A chi-square test was used to determine if the number of pigs with diarrhea was different among groups. All tests were assessed at a confidence level of 95%; the results were considered significant if  $P < .05$ .

## Results

Pigs in Group 1 (negative controls) did not develop diarrhea (Table 1). In the three groups receiving the *E. coli* challenge, diarrhea was noted in some pigs as early as 6 hours postchallenge. In all three challenged groups, some pigs showed no clinical signs of gastrointestinal disease (4, 3, and 5 of 12 pigs in each of Groups 2, 3, and 4, respectively). These pigs appeared bright and alert throughout the 36-hour postchallenge period. Some pigs in all three challenged groups were euthanized before the end of the trial because of severe watery diarrhea

**Table 1: Prevalence of diarrhea and mortality<sup>1</sup> in pigs weaned at approximately 22 days of age, challenged 3 days later with K88<sup>+</sup> *Escherichia coli*, and treated with egg-yolk antibodies (IgY)<sup>2</sup>**

Treatment group <sup>3</sup>	n	Diarrheic pigs	Mortality
1 Negative control	12	0	0
2 Positive control	12	8	2
3 Low dose	12	9	3
4 High dose	12	7	2

<sup>1</sup> Pigs with severe watery diarrhea were euthanised < 36 hours postinoculation.

<sup>2</sup> IgY product obtained from Dr Ron Marquardt, Department of Animal Science, University of Manitoba, Winnipeg, Manitoba, Canada.

<sup>3</sup> Group 1 and 2 pigs were fed unmedicated diet. Group 1 pigs were not challenged. Pigs in Groups 2, 3, and 4 were challenged with 5 mL of a suspension containing 10<sup>11</sup> CFU per mL of a K88<sup>+</sup> *E. coli*. Groups 3 and 4 were treated with IgY product mixed with the same diet fed to Groups 1 and 2. The ration contained 0.32% IgY product (dose recommended by the manufacturer) for Group 3 pigs and 3.2% IgY product (10 × the recommended dose) for Group 4 pigs.

and dehydration (2, 3, and 2 of 12 pigs in Groups 2, 3, and 4, respectively). Necropsy examination of pigs with diarrhea revealed fluid-filled intestines and enteritis consistent with colibacillosis. There were similar levels of diarrhea in each of the challenged groups (chi-square test = 0.75, *P* < .69).

Mean titer of egg-yolk-antibody activity in the feed with 10 times the recommended level of product (Group 4) was 0.03, and for the lower level of inclusion (Group 3) the titres averaged 0.003.

Titers of IgY in stomach and intestinal content from both treated pigs (Groups 3 and 4) and pigs that had received no egg yolk (Groups 1 and 2) were extremely low and did not differ (*P* > .05).

The culture of feces from pigs with diarrhea revealed a pure growth of K88<sup>+</sup> *E. coli*.

## Discussion

Results of this study indicate that the egg-yolk-antibody product did not protect susceptible pigs from developing diarrhea as a result of oral challenge with approximately 5 × 10<sup>11</sup> CFU of K88<sup>+</sup> *E. coli*. Pigs that did not develop diarrhea possibly did not have intestinal receptor sites for K88<sup>+</sup> *E. coli* and therefore were safe from infection.<sup>8</sup> In future studies, it would be useful to establish the presence or absence of K88 receptors in the study pigs. Because the pigs were randomly assigned to treatment, it is likely that similar numbers of pigs in each group had K88 receptors.

It is possible that the challenge dose was

too great, and that the egg-yolk product might have provided protection if a lower dose of *E. coli* had been used. However, some pigs that developed diarrhea were not severely sick, suggesting that the challenge was reasonable.

In other research studies, levels of challenge similar to the one used in this study have been used. Marquardt et al (1999)<sup>9</sup> challenged 3-week-old pigs with 5 mL of *E. coli* at a dose of 10<sup>12</sup> CFU, provided them with 0.5 g of egg-yolk-antibody product three times daily for 2 days after the experimental infection, and demonstrated a positive effect. Pigs in Group 3 of our study consumed similar levels of egg-yolk product, and it is unclear why the results differed. The effect of egg-yolk antibodies appears to be dose dependent. Yokoyama et al (1997)<sup>10</sup> found that 5.5 g of egg-yolk powder per pig per day was protective, whereas 3.5 g was not. Imberechts et al (1997)<sup>11</sup> used 30 g of egg-yolk powder per day to prevent postweaning diarrhea. The pigs in our study consumed less than 400 g of feed per day, ie, less than 13 g of egg-yolk product per day for those in the high-dose category.

The levels of egg-yolk antibody product commonly used in the feed industry in Ontario are 0.1 to 0.2% (1 to 2 kg per tonne). At 1 kg of product per tonne of starter ration, the extra cost per tonne is about \$80 (all costs reported in \$US). According to Marquardt and Li (2001),<sup>3</sup> a pig on average will consume a total of approximately 4 kg of feed during the normal feeding period (ie, 18 to 28 days of age), or about 4

g of egg yolk product at the 0.1% inclusion rate (\$0.32 per pig). The higher dosage of 3.2% used in this study is probably close to the limit of what is economically feasible.

In this study, no appreciable levels of anti-K88 *E. coli* IgY were detected in the gastrointestinal tract, even in pigs on the higher dosage level of the egg-yolk-antibody product. It is possible that the reason for failure of the product to control *E. coli* diarrhea was that the level of egg-yolk antibody in the gastrointestinal tract was too low to be effective.

In studies involving younger weaned pigs (weaned at 10 days of age), egg-yolk antibodies appeared to provide protection against K88<sup>+</sup> *E. coli*.<sup>12</sup> It is possible that in such young piglets the gastric pH is relatively high, and digestive enzymes are not as fully developed as in the 3- to 4-week-old pigs used in this trial. Some researchers have suspected that avian immunoglobulin may be destroyed by the pH and enzymes of the pig's stomach and small intestine and have advocated coating the egg-yolk product to improve its survival.<sup>13,14</sup> However, coating the product or increasing the levels of product incorporated into the feed in order to avoid destruction in the gastrointestinal system is likely to make the use of egg-yolk antibodies prohibitively expensive.

## Implications

- Under the conditions of this study, a chicken egg-yolk-antibody product was not effective in controlling postweaning *E. coli* diarrhea.
- Clinical disease can be produced in 3- to 4-week-old pigs by challenging them with K88<sup>+</sup> ETEC at a dose of 5 × 10<sup>11</sup> CFU.

## References

1. Amezcua R, Friendship RM, Dewey CE, Gyles CL, Fairbrother JM. Presentation of postweaning *E. coli* diarrhea in southern Ontario, prevalence of hemolytic *E. coli* serogroups involved and their antimicrobial resistance patterns. *Can J Vet Res*. 2002;66:73-78.
2. Friendship RM, Dewey CE, Amezcua R, Chernysheva L, Gyles C. Failure of non-antibiotic treatments for the control of postweaning diarrhea. *Proc IPVS Cong*. Ames, Iowa. 2002:234.
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